

Reduction in Leaf Growth and Stomatal Conductance of Capsicum (*Capsicum annuum*) Grown in Flooded Soil and Its Relation to Absciscic Acid

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ABSTRAK

Pengaruh banjir ke atas pertumbuhan daun, kaitan air, perubahan stomata dan kandungan asid absisik (ABA) pada tanaman cili (*Capsicum annuum* L.) di kaji di bawah keadaan persekitaran terkawal. Kebanjiran tanah mempercepatkan penutupan stomata dan pengurangan perkembangan daun tanpa pengurangan defisit air daun. Pengurangan potensi air daun tidak berlaku selama 4 hari rawatan banjir. Seterusnya, potensi air daun berkurangan kepada tahap minima. Kepekatan asid absisik di dalam xilem bertambah selepas 24 jam banjir, dan bertambah secara progresif dengan masa rawatan banjir. Kepekatan asid absisik di dalam daun dan bunga lebih tinggi pada pokok yang di dedahkan kepada banjir di bandingkan dengan pokok yang di beri pengairan berterusan. Dalam kedua-dua keadaan persekitaran, kandungan asid absisik di dalam bunga adalah lebih tinggi daripada daun.

ABSTRACT

The effects of soil flooding on leaf growth, water relations, stomatal responses and absciscic acid (ABA) content in young capsicum (*Capsicum annuum* L.) plants were studied under controlled environmental conditions. Soil flooding induced early stomatal closure and leaf growth reduction without any reduction in leaf water deficit. The undetectable changes in leaf water potential of plants grown in flooded soil persisted for 4 d. Thereafter, leaf water potential was reduced to the minimum values. Xylem sap absciscic acid concentration was increased after 24 h of soil flooding, and increased rapidly with duration of flooding. Plants grown in flooded soil had higher concentration of absciscic acid in leaves and flowers than the well watered plants. Under both conditions, absciscic acid concentrations was higher in flowers than in leaves.

INTRODUCTION

Soil flooding may result in many detrimental morphological and physiological changes, such effects having been reported on many horticultural crop species (Schaffer *et al.* 1992; Else *et al.* 1995). Capsicum (*Capsicum annuum* L.) is considered a flood-sensitive vegetable species as the reduction in stomatal conductance and photosynthesis rate occurs after 24 h of soil flooding (Pezeshki and Sundstrom 1988). Many plant species respond to flooding much as they do to

drought through reduced growth, chlorosis and subsequently leaf senescence (Hurng *et al.* 1994).

Reid *et al.* (1991) indicated that flooding of roots causes variable and complex changes in plant water relations. The reports on the changes in the water relations of plants exposed to root hypoxia are contradictory. Some reports suggest the reduction in stomatal conductance can be related to the leaf water deficit by the effects of flooding (Wadman-van-Schravendijk and van Andel 1986; Sanchez-Blanco *et al.* 1994). In

contrast, other reports suggest that flooding induced stomatal closure prior to alteration in leaf water potential (Andersen *et al.* 1984; Zhang and Davies 1986; Smit *et al.* 1989; Neuman and Smit 1991). Everard and Drew (1989) suggested that a reduced capacity of water flow through roots of herbaceous plants deprived of O_2 is only a partial explanation for flood-induced reduction in leaf growth and stomatal closure. In cases where leaves respond independently to the changes in internal water deficit, a chemical signal is considered to mediate the response. There have already been extensive studies on the action of ABA as a chemical signal that induces stomatal closure of flooded plants (Zhang and Davies 1987; Jackson and Hall 1987), but there is still controversy over whether only ABA or other chemical signals mediate plant responses.

The present study examined changes in leaf growth, water relations and stomatal conductance influenced by soil flooding and determined the role of xylem sap ABA in regulating leaf responses in flooded capsicum plants. The effect of flooding on ABA content in leaves and flowers was also examined.

MATERIALS AND METHODS

Seeds of capsicum (*Capsicum annuum* L.) cv Bell Boy were germinated and raised under glass-house conditions at the Department of Biological Science, University of Lancaster, England during summer 1994. Seedlings were transferred to pots containing 3.3 l of John Innes II compost. At the reproductive stage, plants were transferred into a growth cabinet at a temperature of 22-25°C (day) and 18°C (night), relative humidity of 48% and photoperiod of 14 h with photo flux density averaging 320 ($\text{mol m}^{-2} \text{s}^{-1}$). The plants were allowed to grow for 7 days in the growth cabinet prior flooding treatments. The plants were randomly divided into two groups with five replicates each of 3 plants. Control plants were watered daily to the drip point. Another group of plants was flooded by closing the drainage holes of the plastic container. Flooding was maintained at 3 cm above the soil surface. The surface of each pot was covered with black polythene plastic in order to reduce evaporation.

Over the following 6 days, measurements of leaf length, leaf breadth, stomatal conductance, leaf water potential and abscisic acid (ABA) in xylem, leaf and flower were made.

Leaf Length and Breadth

The length and breadth of the youngest leaf from each plant was measured and tagged prior to flooding treatments. On every sampling date, leaf expansion was recorded by measuring the differences between the measured and the initial leaf length and breadth values.

Stomatal Conductance

Stomatal conductance was determined on the abaxial surface of the youngest fully expanding and mature (4th leaf from top canopy) leaves on four plants, using a diffusion porometer (AP4, Delta-T Devices Ltd., Cambridge, UK). Measurements were performed at 4 h into the light period.

Leaf Water Potential

Leaf water potential was determined after measuring stomatal conductance on the same leaf used for stomatal conductance. Leaf water potential was measured using a pressure chamber (Soil Moisture Equipment Co., Santa Barbara, CA, USA).

Estimation of Absciscic acid Concentration

ABA concentration was estimated in leaves, flower and xylem sap using a radioimmunoassay technique (RIA). Samples of leaves and flowers were placed in open plastic vials, wrapped in aluminium foil and frozen in liquid nitrogen. They were then freeze dried, finely ground and extracted overnight at 5°C with distilled, deionised water. Samples were extracted using a ratio of 1:25 (leaf dry weight:solvent volume). Xylem sap was collected by pressurizing the cut stem portion of the plants. The extruded sap was collected in a capillary tubing and transferred to eppendorf vials, and then frozen in liquid nitrogen. Analysis of ABA was carried out using monoclonal antibody (McAb) that is specific for (+)-ABA (AFR MAC62) (Quarrie *et al.* 1988).

RESULTS

Leaf expansion, measured as leaf length and breadth, was markedly reduced with soil flooding; a greater reduction was observed for leaf length. As illustrated in *Fig. 1*, leaf length shows greater differences after day 1 of plants in the treatment. For the first 3 days of soil flooding, leaves on soil-flooded plants elongated at an aver-

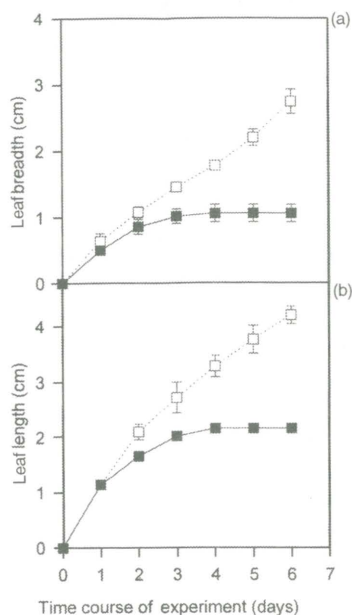


Fig 1. Cumulative leaf length and breadth of capsicum plants when subjected to soil flooding ■ or well watered □ conditions. Bars represent \pm SE means of 5 replicates. Bars were not indicated if less than SE values.

age rate of approximately 0.8 cm d^{-1} compared with 0.6 cm d^{-1} for leaves grown in soil flooding. After day 3, the differences were exacerbated when leaf expansion ceased in flooded plants.

Fig. 2 shows that soil flooding induced early stomatal closure irrespective of leaf age. After 2 days of soil flooding, stomatal conductance in both young and old leaves had declined to less than $100 \text{ mmol m}^{-2} \text{ s}^{-1}$. Further exposure to soil flooding caused the stomatal conductance to decline to minimum values on day 3 and day 5 on old and young leaves, respectively. As illustrated in Fig. 2b, leaf water potential of flooded plants began to decline after day 4 of soil flooding, suggesting evidence of non-hydraulic factors that regulate the reduction in leaf expansion and stomatal conductance. We observed no symptoms of leaf wilting on plants in the early hours of flooding, which failed to suggest the occurrence of transient water stress on rapidly transpiring plants when immediately exposed to the oxygen deficient environment. After day 4, leaf water potential declined rapidly to reach a minimum value of approximately -1.2 MPa by day 6.

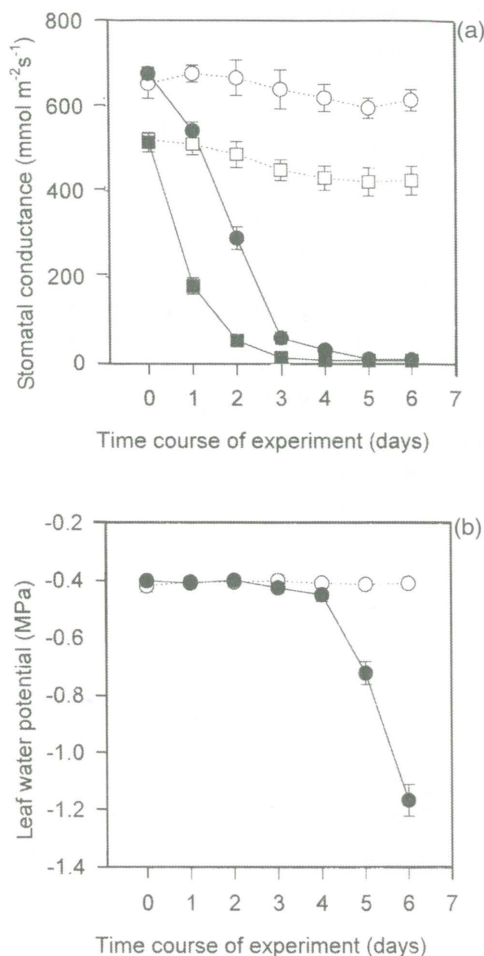


Fig 2 Stomatal conductance and leaf water potential of mature capsicum plants when exposed to soil flooding (closed symbol) and well watered (open symbol). For stomatal conductance; ○ young, expanding leaves and □ old leaves. Bars represent \pm SE means of 4-5 replicates. Bars were not indicated if less than SE values.

When plants were exposed to soil flooding, xylem sap ABA increased by day 1, and then increased progressively with the duration of flooding (Fig. 3). Similarly, there was an increase in ABA concentration in leaf extrudate, which was apparent by day 3 of soil flooding. The ABA concentration in flower extrudate was also increased with 5 days of soil flooding. The concentration of ABA was 1.5-2.3 times higher in flowers than in leaf extrudates under flooded conditions (Fig. 4).

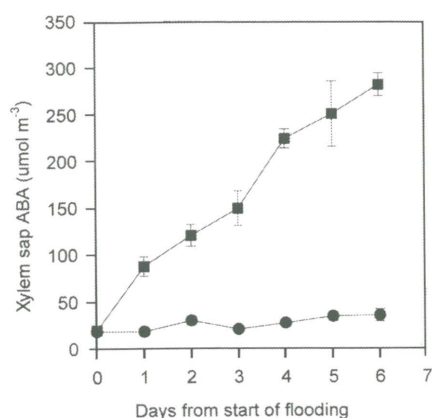


Fig 3. Xylem sap ABA in flooded (■) and well watered (●) capsicum plants. Bars represent + SE of 4 replicates.

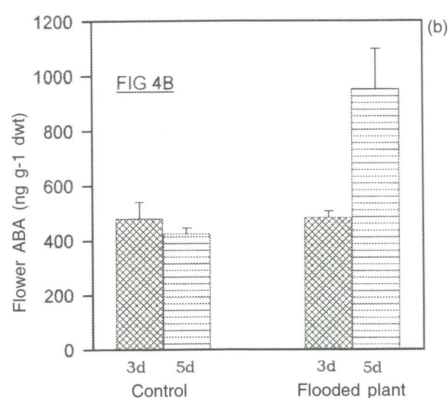
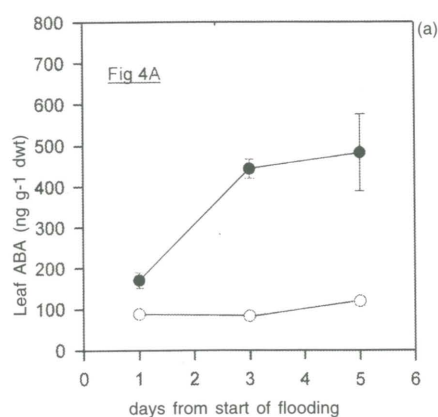


Fig 4. ABA in leaf (Fig 4a) and flower extrudate (Fig 4b) of capsicum plants when exposed to soil flooding. In Fig 4a ○ = well watered and ● = flooded plants. Bars represent ± SE means of 4 replicates.

DISCUSSION

Soil flooding resulted in reduced leaf expansion, by a reduction in leaf length and breadth. There was apparently no leaf expansion after 2 days of soil flooding. There was, however, no senescence of leaves on flooded capsicum plants throughout the experimental period. Smit *et al.* (1989), working with poplar plants, reported that rate of leaf expansion declined within 8 h and was suppressed for the duration of the experiment. Their studies also suggested that leaf growth reduction was due to the changes in cell wall extensibility rather than turgor pressure. Neuman and Smit (1991) reported the pattern of leaf length during 4 h of flooded *Phaseolus vulgaris* followed 4 different phases: (i) an immediate decline in the rate of elongation; (ii) transitory leaf shrinkage; (iii) a period of no growth followed by (iv) growth resumption at a reduced rate relative to prehypoxia. In this study, the reduction in leaf growth was only apparent after 24 h of flooding. The reduction in leaf growth relative to the control may have occurred immediately in the present study. Measurements using a differential transformer, which was not done in this study, might detect immediate changes in capsicum plants.

The present study showed no reduction in leaf water potential within 4 days of flooding. The results suggest flooding reduced leaf growth independently to the development of internal water deficit. Davies *et al.* (1987) suggested that when leaf water relations are unaffected by root hypoxia, another mechanism might be involved in affecting the metabolic effect in the roots. Flooding induced a progressive decrease of stomatal conductance in both young and old leaves. By day 3, stomatal conductance values were very low in flooded plants on both leaves. Pezeshki and Sundstrom (1988) showed a similar trend in early reduction in stomatal conductance in capsicum. There are, however, uncertainties on the role of water relations affecting the stomatal response. There are reports which indicate that stomatal closing in response to flooding occurred both in the presence and in the absence of water deficit (Jackson and Hall 1987). The role of water deficit is considered transient and the shoot regains its water balance within 24 h (Bradford and Hsiao 1982; Everard and Drew 1989; Else *et al.* 1995). Schildwacht (1989) suggested that during the first hour of root anoxia, the leaf elongation rate was

reduced as a consequence of the lower water potential. Thereafter, however, elongation rates were lower than would be expected on the basis of the plant water relations. The changes during the first few hours of flooding were not determined, but the results are consistent with the earlier findings on the overall changes in leaf growth and also stomatal conductance independent of plant water relations.

Since soil flooding initially affects the roots, it is possible that a chemical signal may be transmitted to the leaves which then triggers stomatal closure or reduced leaf growth. The mechanism of non-hydraulic factors causing the above-mentioned responses has been studied extensively. Because leaves respond to soil flooding independently of the changes in water flux, there could be a signalling mechanism between roots and leaves. There is considerable evidence of ABA as one of the likely candidates in the non-hydraulic messages that regulate leaf responses. The results from the present study may support the role of ABA in mediating the leaf responses. There was a four-fold increase in xylem sap ABA in flooded plants by day 1, which increased progressively with the duration of flooding. Similar to the observations of Jackson and Hall (1987), the increase in ABA was not preceded or accompanied by loss in leaf turgor. Neuman and Smit (1993) showed that ABA has no role in regulating leaf growth in poplars. They found only a small and transient increase in leaf ABA in poplars with hypoxic roots, and endogenous ABA in the xylem sap was found to be much lower than the concentration required to mimic the leaf response to root hypoxia. The discrepancies may be due to species and environmental differences. The possibilities of other chemical signals causing reductions in leaf growth and stomatal conductance to plants were not excluded exposed to flooding, however, were not excluded. Reid *et al.* (1991) suggested that ABA is unlikely to act alone as the effects of other phytohormones on stomatal functioning such as the existence of non-ABA in non flood-induced elevations of ethylene and lowered cytokinins. Else *et al.* (1995) found a greater involvement of ethylene precursor 1-aminocyclopropane 1-carboxylic acid (ACC) within 24 h of soil flooding rather than ABA or nitrate in tomato plants.

The present study shows an elevated ABA concentration in flower extrudates of flooded

plants on day 5 (Fig. 4). This could be associated with the observation of wilting on the flowers of flooded plants which are about to abscise from the plants. This is consistent with ABA's role in the senescence of flowers in oranges (Talon *et al.* 1990) in lupin (Porter 1977) and in olives (Kitsaki *et al.* 1995). Dunlap and Robacker (1990) reported that ABA is transported to senescing flowers. The higher concentration of ABA in flowers than in the leaves in both control and flooded plants suggests that leaves act as organs of ABA biosynthesis and flowers as ABA receptors (Hein *et al.* 1984).

In conclusion, the results of this investigation provide circumstantial evidence that the reduction in leaf growth and stomatal responses in capsicum plants is mediated to the increase in ABA concentration in the xylem sap. Detailed investigations on whether delivery rate or concentration of ABA produced a greater effect in flooded plants are needed.

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